REMARKS

The withdrawal of certain prior ground of objection and rejection, as set forth at paragraphs 6-9, is noted with appreciation.

The present claims are directed toward modified Fab' fragments to which at least one effector molecule is attached, characterized in that the heavy chain in the fragment is not covalently bonded to the light chain, and further in that both the interchain cysteine of C_L and the interchain cysteine of C_H1 have been replaced with another amino acid, and the hinge region contains one or two cysteines.

Amendment of the Claims

Claim 1 is amended herein to recite that at least one effector molecule is a 5,000 to 40,000 KDa PEG or a PEG derivative. This amendment finds support in the specification as originally filed at page 8, lines 8-9. Claim 1 is amended herein to recite that the derivative can be selected from an α-halocarboxylic acid or ester, an imide, a vinyl sulphone or a disulphide. This amendment finds support in the specification as originally filed at page 8, lines 1-4. Claim 1 is amended herein to recite that the hinge region is optionally modified. This amendment finds support in the specification as originally filed at page 4, lines 14-16, where it is stated that a modified hinge region is any hinge that differs in length and/or composition from a native hinge region; and is further supported at page 4, line 17 – page 5, line 8, which gives examples of hinge region modifications.

Claims 27 and 28 are canceled.

Claim rejections – 35 U.S.C. § 102

The rejection of claims 1- 7, 10-18, and 27-30 as anticipated by Humphreys WO 99/15549 (hereinafter "Humphreys '549") as evidenced by Rodrigues is respectfully traversed. Humphreys '549 is concerned with the production of dimeric $F(ab')_2$ fragments containing the hinge sequence TCPPCPXYCPPCPA, i.e. a hinge sequence having four cysteines. Humphreys '549 also describes the PEGylation of such fragments (Claim 8 and P.24 lines 8-24 (PEGylation of a $F(ab')_2$ containing hinge 2o 'TSDKTHTCPPCPATCPPCPA')). Thus, the over-riding emphasis in

Humphreys '549 is on dimeric $F(ab')_2$ fragments with a hinge sequence containing **four** cysteines.

In maintaining the 102 rejection, the Examiner states at page 4 of the Office Action:

> [Humphreys teaches] antibody fragments comprising a Fab or Fab' wherein the interchain cysteines of the CL and CH1 have been mutated to serines and Humphreys teaches various hinge peptides comprising one, or two or more cysteines (e.g., sequences identical to SEQ ID Nos.: 1-3) for the attachment of effector molecules, including polyethyleneglycol (PEG) (e.g., see entire document, particularly Table II, pp. 3-6, 8-11 and Examples).

These statements are respectfully traversed. In Example 1, Humphreys '549 teaches the production of di-Fab' in E.coli using different hinge sequences. The di-Fab' fragments are made from Fab' constructs. To minimize any possible incorrect interchain disulphide bonds between hinge regions and any other cysteines, the interchain disulphide bond was removed. PCR mutagenesis was used to change the interchain cysteines of cKappa and C_H1 to serines (p. 12), and the interchain Cys codon in the light chain (LC) cKappa was changed by PCR mutagenesis to Ser (Example 1 at p. 13). After these mutations were complete, the Fab' fragments were used to make di-Fab' fragments, and the production of the di-Fab' fragments was evaluated. Only *after* the two fragments were joined to make the di-Fab' fragment was an effector molecule added, such as by PEGylation (p. 24). Humphreys '549 does not disclose a Fab' fragment with an effector molecule. In Humphreys '549 the disclosed Fab' fragments have but a single purpose, i.e., to act as precursors for di-Fab' fragments. The individual Fab' fragments are not disclosed as having any separate utility, and they are never disclosed as being PEGylated, or as having any other effector molecule attached.

Furthermore, in Humphreys '549 only those di-Fab' fragments having four cysteines in the hinge region were used for the attachment of effector molecules. At page 21, lines 36-39 of the Humphreys '549 reference, it is explained that "hinge ½" has one cysteine, "hinge 1" has two cysteines, and "hinge 2o" has four cysteines. As stated at page 24 of the reference, only the sample with "hinge 2o" having four cysteines was PEGylated. Thus, contrary to the statement in the Office Action, the Humphreys '549 reference does *not* disclose Fab' fragments having hinge peptides with only one or two cysteines used for the attachment of effector molecules.

The unstated implication of the Examiner's statement above is that, since Humphreys '549 teaches Fab' fragments with the interchain cysteines mutated to serines, and since Humphreys '549 teaches effector molecules, then Humphreys '549 must teach mutated Fab' fragments with effector molecules. As explained above, that is not the case. *Humphreys '549 does not disclose mutated Fab' fragments with effector molecules. Humphreys '549 discloses the attachment of effector molecules only to di-Fab' fragments, and only then to di-Fab' fragments having four cysteines in the hinge.* Moreover, in Humphreys '549 the only purpose of the mutagenesis of the cysteine to serine is to prevent undesired bonding when the two Fab' fragments are joined to form a di-Fab'. Humphreys '549 discloses no reason why such mutagenesis would be done if the fragments were to be used in their Fab' form.

With respect to the particular portions of the Humphreys '549 reference cited by the Examiner at the bottom of page 4 of the Office Action, pages 3-6 teach polypeptides with four cysteines; at page 5, lines 21-23 it is taught that such sequences are particularly suitable where each sequence can function as a hinge region to provide a dimerisation capacity. Pages 8-11 teach that the desired sequences can be generated in Fab or Fab' fragments manufactured in E.coli. These pages also teach effector molecules. It is noted, however, that Humphreys '549 does not teach effector molecules in the particular molecular weight range now recited in claim 1.

The Rodrigues reference does not overcome the deficiencies of Humphreys '549. The present application teaches at page 10, lines 9-11 that the cysteines to be replaced are at position 214 of the light chain and position 233 of the heavy chain. Rodrigues at p. 6955, second column, teaches mutation to serine of the cysteines located at position 214 of the light chain and position *223*, not 233, of the heavy chain.

Accordingly, Humphreys '549, taken either alone or as evidenced by Rodrigues, does not anticipate the subject matter of claim 1 under 35 U.S.C. § 102. Claims 2-7, 10-18 and 29-30, which depend from claim 1 either directly or indirectly, also are not anticipated. Independent claim 27 contains the same limitations as claim 1, such that claim 27 and claim 28 which depends thereon also are not anticipated. It is respectfully requested that this ground of rejection be withdrawn.

Double Patenting

The obviousness-type double patenting rejection of pending claims 1-7, 10, 15-18 and 27-30 over claims 7 and 10 of Humphreys U.S 6,642,356 (hereinafter "Humphreys '356") in view of Humphreys '549 is respectfully traversed.

The differences between the present application claims and the claims 7 and 10 of Humphreys '356 are many:

- Claim 1 of the present application recites that the hinge region contains one or two cysteines. Claims 7 and 10 of the '356 patent each ultimately depend from claim 1 thereof which recites a peptide that functions as a hinge region, the peptide comprising the amino acid sequence set forth in SEQ ID NO:1. This sequence, which appears at column 27 of the '356 patent, contains four cysteines, one each at positions 2, 5, 9, and 12. Thus, the fact that the claim recites another amino acid at positions 7 and 8 does not affect the number of cysteines in the recited hinge peptide.
- Claim 1 of the present application recites that the heavy chain in the fragment is not covalently bonded to the light chain. This claim element is not recited in any of the claims of the '356 patent.
- Claim 1 of the present application recites that both the interchain cysteine of C_L and the interchain cysteine of C_H1 have been replaced with another amino acid. This claim element is not recited in any of the claims of the '356 patent.
- Claim 1 of the present application has been amended to recite that at least one effector molecule is a 5,000 to 40,000 KDa PEG or PEG derivative. This claim element is not recited in any of the claims of the '356 patent.
- Claim 1 has been amended to recite that the PEG derivative is selected from an α-halocarboxylic acid or ester, an imide, a vinyl sulphone or a disulphide. This claim element is not recited in any of the claims of the '356 patent.

It would not have been obvious to modify claims 7 and 10 of Humphreys '356 to include all the differences noted above, nor would the results of such

modifications have been predictable. Nor does the Examiner state where such a teaching may be found.

At page 7, lines 4-8 of the Office action, the Examiner states:

> ...Humphreys teach Fab and Fab' fragments in which the interchain cysteines of the C_H1 and CL are substituted with serine and the hinge sequence TCPPCPXYCPPCPA, identical to the hinge sequence of U.S. Patent No. 6,642,356 B1, is used for the attachment of one or more PEG molecules....

Again, this statement is respectfully traversed, because Humphreys teaches the use of effector molecules *only* on the di-Fab' fragments, *not* on the Fab or Fab' fragments. Further, the referenced hinge sequence has four cysteines, while the presently claimed Fab' fragments have hinge regions with only one or two cysteines. Humphreys' teaching of PEGylation of a hinge sequence in di-Fab' fragments containing four cysteines does not render obvious the PEGylation of a hinge sequence in a Fab' fragment containing one or two cysteines.

In the paragraph spanning pages 7-8 of the Office Action, the Examiner states that it would have been obvious "... to produce a Fab or Fab' fragment comprising the hinge sequence of SEQ ID NO:1...and wherein the interchain cysteines of the C_H1 and C_L are substituted with serine and the free cysteine thiols of SEQ ID NO:1 are attached to a PEG molecules...." But the instant claimed fragments do not have SEQ ID NO:1 of Humphreys '356 at the hinge; instead, they have hinges that contain only one or two cysteines. Nor does the cited art teach attachment of PEG effector molecules to Fab or Fab' fragments, or the molecular weight range of the PEG effector molecules as now claimed, or that it is valuable to have such fragments wherein the interchain cysteines of the C_H1 and C_L are substituted with serine if the fragments are not to be dimerised.

With regard to proof of unexpected results, the Examiner's attention is directed to the data in the specification as filed which demonstrates that the claimed fragments are unexpectedly stable and retain advantageous affinity properties. In table 2 at page 21 of the application as filed, affinity data is presented for an antibody of the present invention and a control antibody. The antibody g8516 LC-S HC-S hinge CAA has all the features of claim 1, namely, no interchain cysteines and one 40,000 KDa PEG linked to a cysteine in the hinge. The affinity data for the fragment is 0.13 nM. This is comparable to the 0.10nM affinity of the control g8516 LC-C

HC-C, hinge CAA which has a disulphide bond between the heavy and light chains. If the arrangement of the Fab' fragment was altered or destabilized by the omission of the interchain cysteines then this would manifest itself in a reduced affinity. Thus from this data it can be concluded that, surprisingly, the affinity is not adversely affected by the lack of an interchain cysteine.

Table 3 at page 22 of the specification as originally filed shows that the *in vivo* pharmacokinetic data for antibody g8516 LC-S HC-S hinge CAA is very similar to that of the control. This provides evidence that, unexpectedly, the *in vivo* stability of the antibody format of the invention is adequate.

Prior to the applicants' invention herein, it simply was not possible to predict that the presently claimed antibody fragments would be stable *in vivo* and retain the advantageous affinity properties, as demonstrated by the data of the specification. While it was known that antibody fragmenst containing disulphide bonds could be PEGylated, it was not known or suggested that Fab and Fab' fragments could be PEGyated and retain their stability in vivo and affinity.

Accordingly it is respectfully submitted that the present claims are not invalid for obviousness type double patenting over claims 7 and 10 of the '356 patent.

Claim rejections – 35 U.S.C. § 112

Claims 1-18, 27-28 and 30 are rejected as indefinite with respect to the term "derivative." Claim 1 has been amended to recite the particular PEG derivatives encompassed by the claims. It is respectfully submitted that these amendments are sufficient to overcome this ground for rejection.

Claim rejections – 35 U.S.C. § 103

The rejection of claims 1 and 8-9 as obvious over Singh et al. in view of Hsei et al. and Humphreys '549 is respectfully traversed.

As the Examiner correctly notes at page 10, lines 7-14 of the Office Action, Singh et al. do not specifically teach an antibody comprising a Fab' fragment comprising a hinge region containing one or two cysteines and wherein the Fab' fragment has been modified by attachment of at least one PEG or PEG derivative wherein both the interchain cysteine of C_L and the interchain cysteine of C_H1 have been replaced with another amino acid such that the heavy chain in the fragment is not covalently bonded to the light chain.

The Hsei reference teaches nothing about limiting the number of cysteines in the hinge region of a Fab' fragment to one or two, and attaching a PEG molecule to that hinge region.

The Examiner's characterization of the Humphreys '549 reference at the paragraph spanning pages 10-11 of the Office action is respectfully traversed. The Fab' fragments of Humphreys '549 having hinge region peptides comprising one or two cysteines did not "efficiently generate dimers." At page 15, lines 28-30, Humphreys states that the "hinge ½" Fab' (i.e., the hinge containing only one cysteine) produced no detectable di-Fab', and the "hinge 1" Fab' (the hinge containing two cysteines) produced moderate amounts of di-Fab.' Further, the PEG receptor molecules were attached only to those di-Fab' fragments having 'hinge 2o,' i.e., the hinge having four cysteines.

Thus combining these three references would not have led one of ordinary skill in the art to make the subject invention. Humphreys '549 teaches that Fab' fragments having only one cysteine in the hinge region are not good candidates for making di-Fab', and that Fab' fragments having two cysteines in the chain are only moderately effective. Humphreys '549 also teaches PEGylation only of di-Fab' fragments having four hinge cysteines. Hsei teaches modifying the heavy and light chains, but does not teach modifying the hinge region to limit the number of cysteines. Again, contrary to the statement in the middle of page 12 of the Office Action, Humphreys '549 does not teach Fab' fragments in which the hinge regions comprise one or two cysteines *and* that efficiently generate dimers; in fact, Humphreys '549 suggests to one skilled in the art that Fab' fragments with one or two cysteines have no utility and do not merit further consideration, as shown by the fact that these fragments were not PEGylated. Referring to the Examiner's comment at the top of page 13, if the strongest rationale for combining references is a recognition in the art that some advantage or expected beneficial result would have been produced by their combination, then in this case there is no rationale to combine references because Humphreys '549 teaches that there is no advantage to using Fab' fragments with hinge regions containing one or two cysteines, because they do not efficiently form di-Fab' fragments, and Humphreys '549 did not attach effector molecules to them.

Further, none of the cited references, taken alone or together, teaches or suggests that the antibody fragments as presently claimed would retain their stability

in vivo and their affinity, as demonstrated by the data in Tables 2 and 3 of the specification, as discussed above.

As all points of rejection have been overcome, a Notice of Allowance is respectfully requested. The Examiner is invited to contact the undersigned applicants'' representative if it is believed that such contact would further the prosecution of this application.

Respectfully submitted,

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